RNA INTERFERENCE TARGETING INTERSTITIAL COLLAGENASE IS A POTENTIAL THERAPEUTIC TOOL TO TREAT PSORIASIS

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Matrix metalloproteinases play an important role in maintaining skin homeostasis, promote wound healing, and are involved in triggering inflammation. They are implicated in the structural changes occurring in the epidermis of psoriatic patients and also facilitate infiltration of the skin by immune cells by regulating permeability of dermal capillaries. In this light, control over the enzymatic activity of matrix metalloproteinases is crucial for a successful treatment outcome in patients with psoriasis. The aim of this work was to investigate the effect of RNA interference on the progression of psoriasis by targeting interstitial collagenase of epidermal keratinocytes. As part of the experiment, the latter were transduced with lentiviral particles that encode small hairpin RNA. Gene expression was measured by real time polymerase chain reaction. Enzymatic activity was measured by zymography. RNA interference was found to lead to a 20- and 4-fold decrease in the expression and enzymatic activity of interstitial collagenase, respectively. Expression of homologous genes (*MMP2*, *-9* and *-12*) changed insignificantly. In contrast, there were marked changes in expression of cytokeratin (*KRT1*: 16.89 ± 0.97; *KRT14*: 2.36 ± 0.19; *KRT17*: 0.12 ± 0.01; *KRT18*: 0.56 ± 0.02), involucrin (0.79 ± 0.11) and filaggrin (6.99 ± 0.97). Besides, RNA interference caused a significant decline in cell migration rates, although it did not affect cell proliferation. Thus, small hairpin RNAs targeting interstitial collagenase are potentially therapeutic for psoriatic patients due to their ability to regulate expression of genes implicated in psoriasis (*IVL*, *FLG*, *KRT1*, *-14 -17*, and *-18*).

Keywords: psoriasis, interstitial collagenase, lentiviruses, transduction, transfection, small hairpin RNA, cytokeratins, involucrin, filaggrin

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ОЦЕНКА ТЕРАПЕВТИЧЕСКОГО ПОТЕНЦИАЛА РНК-ИНТЕРФЕРЕНЦИИ ИНТЕРСТИЦИАЛЬНОЙ КОЛЛАГЕНАЗЫ ДЛЯ ЛЕЧЕНИЯ ПСОРИАЗА

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Матриксные металлопротеиназы играют важную роль в поддержании гомеостаза кожи, заживлении ран и инициации воспалительного процесса. При псориазе матриксные металлопротеиназы участвуют в структурных перестройках эпидермиса и регуляции проницаемости микрокапилляров дермы, способствуя инфильтрации кожи клетками иммунной системы. В силу этого умение контролировать активность матриксных металлопротеиназ представляется необходимым для успешного лечения псориаза. Целью данной работы являлась оценка возможных изменений в патогенезе псориаза в результате РНК-интерференции интерстициальной коллагеназы в эпидермальных кератиноцитах. Для этого клетки трансдуцировали лентивирусными частицами, кодирующими в геноме малую интерферирующую РНК. Для анализа уровней экспрессии генов использовали метод полимеразной цепной реакции в режиме «реального времени». Ферментативную активность определяли методом зимографии. Согласно полученным результатам РНК-интерференция привела к снижению уровня экспрессии гена и ферментативной активности интерстициальной коллагеназы в 20 и 4 раза соответственно. При этом экспрессия гомологичных генов (*MMP2*, *-9* и *-12*) менялась незначительно. Напротив, нами были показаны изменения в уровнях экспрессии генов цитокератинов (*KRT1*: 16,89 ± 0,97; *KRT14*: 2,36 ± 0,19; *KRT17*: 0,12 ± 0,01; *KRT18*: 0,56 ± 0,02), инволюкрина (0,79 ± 0,11) и филаггрина (6.99 ± 0,97). Помимо этого, РНК-интерференция вызвала существенное снижение скорости миграции клеток, хотя практически не повлияла на их пролиферацию. Таким образом, терапевтический потенциал малых интерферирующих РНК, специфичных к интерстициальной коллагеназе, заключается в нормализации экспрессии важных для патогенеза псориаза генов (*IVL*, *FLG*, *KRT1*, *-14 -17* и *-18*).

Ключевые слова: псориаз, интерстициальная коллагеназа, лентивирусы, трансдукция, трансфекция, малая интерферирующая РНК, цитокератины, инволюкрин, филаггрин

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Psoriasis is one of the most common skin diseases [1]. The hallmarks of plaque psoriasis, which is accountable for 90 % of the reported cases, are the raised and clearly delimited epidermal lesions and chronic skin inflammation that leads to an infiltration of the skin by immune cells. At the molecular level, psoriasis is characterized by differential expression of genes encoding cytokeratins, involucrin and filaggrin, which are involved in the differentiation of epidermal keratinocytes. Particularly, the expression of *KRT1* and *-14* elevates, whereas the expression of *KRT17* and *-18* declines.

Unfortunately, there is no cure for psoriasis. On the other hand, the available antipsoriatic treatments can clear psoriasis, often for long periods of time. In this respect, the development of new therapeutic approaches for psoriasis will remain a high priority task that requires an immediate action. In turn, a search for effective treatments will not be successful without understanding how psoriatic plaques are developing and how their progression can be manipulated. Accomplishing this task also requires identifying the key participants of the disease pathogenesis and revealing their role in the disease progression.

In the lab, our research is focused on interstitial collagenase (IC). In psoriasis, IC is plays an important role in epidermal remodeling, interactions between the epidermal keratinocytes and permeabilization of dermal microcapillaries to the immune cells [2]. Moreover, the expression levels of certain matrix metalloproteinases, including IC, correlate with the severity of the disease and they typically elevate when psoriasis flares. In this respect, it would be important for us to find out how to control metalloproteinase activity in the lesional skin.

Unfortunately, the idea that the activity of individual matrix metalloproteinases can be blocked by specific inhibitors is failed. To date, the clinical data suggest that the proposed experimental treatments either have a poor efficiency [3] or produce life-threatening adverse effects [4]. However, it is quite possible to suppress the individual matrix metalloproteinase genes by knocking them down with specific small hairpin RNAs (shRNAs). In the other words, lesional skin can be treated with certain substances that are capable to destroy mRNAs transcribed from a particular gene. For instance, this kind of experimental treatment could contain nonpathogenic viral particles that encode a target-specific shRNA [5, 6].

According to the published data, the transfection efficiency is not the same in different cell lines. Moreover, it may still sufficiently vary even when the same protocol is used. For instance, HaCaT cells, which are epidermal keratinocytes, are more difficult to transfect or transduce than HEK293, which are human embryonic kidney epithelial cells [7]. A relatively low transfection and transduction efficiency of HaCaT cells can be explained by at least two reasons. First, although HaCaT are immortalized cells, they have a protective mechanism that defends them against foreign RNA. Particularly, HaCaT express fully functional receptors TLR3 and TLR7 that recognize viral RNA [8, 9]. Second, HaCaT originate from epidermal keratinocytes that form a protective skin barrier. This protective barrier prevents a penetration of the body by microorganisms and chemical agents. Moreover, this barrier protects the body from environmental hazards, such as UV and ionizing radiation. In this respect, it is not surprising that HaCaT are more resistant to the transfection reagents, compared to the other cells lines, which are originated from the internal organs [10]. The later also explains why the delivery of viral shRNA into primary cells and the skin cells in particular looks for us as a very challenging task.

In this paper, we demonstrated how IC silencing in human epidermal keratinocytes may affect the pathogenesis of psoriasis.

METHODS

Cell culturing

Cells were cultured in the DMEM medium supplemented with L-glutamine (PanEco, Russia), antibiotic-antimycotic and 5 % fetal bovine serum (Thermo Fisher Scientific, USA). The medium was replaced every other day. Once the cells had reached 70–75 % confluence, they were reseeded into new dishes, one fifth of the collected cells per dish. The cells were counted in hemocytometer. To measure the areas covered by the cells and estimate the ratio of fluorescent cells, the images of transduced cells were analyzed with ImageJ software (NIH, USA) using “Freehand selection” tool and “Cell counter” plugin, respectively.

Cell transfection and transduction

To obtain genetically modified virions that encode necessary shRNA, the packaging cell line HEK293 was cotransfected with 4 plasmids: pMDLg-pRRE, pREV-TRE, pCMV_VSV_G and pGPV. Plasmids pMDLg-pRRE, pREV-TRE, and pCMV_VSV_G, which encoded viral genes necessary for assembling the virions, were a generous gift of Prof. M. A. Lagarkova

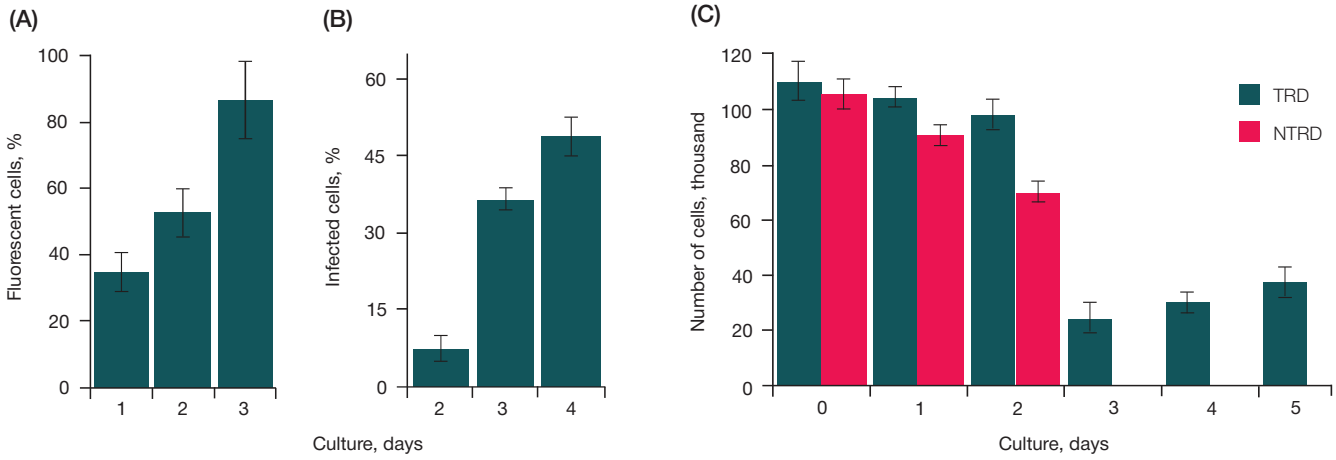

Fig. 1. Lentiviral transduction. **(A)** Ratio of HEK293 cells emitting the fluorescent light in the course of transfection. **(B)** Ratio of HaCaT cells emitting the fluorescent light in the course of transduction. **(C)** Number of viable HaCaT cultured in the presence of puromycin. TRD — transduced cells; NTRD — non-transduced cells (negative control). Transfection and transduction were performed as described in *Methods*

(Vavilov Institute of General Genetics, RAS). The lentiviral vectors pGPV-17019250-MMP1 and pGPV-17019250-CTR encoding shRNAs were obtained as described earlier [11, 12]. Vector pGPV-17019250-MMP1 was used to obtain the IC-deficient cells (HaCaT-IC), whereas vector pGPV-17019250-CTR was used to obtain control cells (HaCaT-CTR) that expressed scrambled shRNA. Moreover, each pGPV vector also encoded puromycin-resistance factor (*PuroR*) and green fluorescent protein (*copGPF*). These genes were necessary for a selection of transduced cells on puromycin selective medium. Before the experiment, a mix of the plasmids mentioned above (10 : 5 : 2 : 10) was diluted in unsupplemented DMEM .Then, the transfection reagent Metafectene (Biontex, Germany) was added and the obtained aqueous solution was incubated for 15-minutes at room temperature. At the end of the incubation, the solution was added to HEK293 cells (30–40 % confluence). In 6 hours, the culture medium was replaced with a fresh one. Then, four days following the transfection, a virion-containing medium was collected, filtered (pore diameter — 0.4 μm) and used to transduce HaCaT cells.

The transduction of human epidermal keratinocytes HaCaT (<60 % confluence) was performed for 4 days on a daily basis by replacing their own culture medium with virion-containing medium collected from HEK293. In four days, the transduced cells were transferred to the medium contained 5 μg/ml puromycin (Thermo Fisher Scientific) and cultured there for a week to select the puromycin-resistant cells with different levels of IC expression.

Preparation of cell homogenates

To obtain cell homogenates, the cells were harvested at 60–70 % confluence and resuspended in the RIPA buffer (25 mM Tris, 150 mM NaCl, 0.1 % sodium dodecyl sulphate, 0.5 % sodium deoxycholate, 1 % NP-40, pH 7.4) (Thermo Fisher Scientific) for 1–2 min at 2–8 °C to achieve a complete degradation of the cells (500 μl of the buffer per T-25 flask).

Protein assay

The protein concentration was measured using the fluorimetric Qubit Protein Assay Kit (Thermo Fisher Scientific) according to the manufacturer's protocol.

Polyacrylamide gel electrophoresis and zymography

Polyacrylamide gel electrophoresis was performed according to Laemmli [13, 14]. The acrylamide concentrations in resolving and stacking gels were 10 % and 5 %, respectively. Zymography was performed in 10 % polyacrylamide gel containing 4 mg/ml collagen (Thermo Fisher Scientific) as described earlier [15]. Briefly, to prepare the gel, the gel components were mixed on an ice bath at 2–8 °C and transferred to room temperature for polymerization. The IC activity was assessed by densitometry using the plugin "Gels" for ImageJ software (NIH).

Extraction of total RNA

Total RNA was extracted with TRIzol reagent (Thermo Fisher Scientific) as described earlier [16]. Quality of the obtained RNA samples was verified using non-denaturing 1.5 % agarose gel electrophoresis. The RNA concentration was measured using the fluorimetric Qubit RNA BR Assay Kit (Thermo Fisher Scientific) according to the manufacturer's protocol.

Real-time PCR

Before the experiment, total RNA was converted to cDNA using MMLV RT kit (Evrogen, Russia) according to the manufacturer's protocol. The primer sequences were obtained from the Probe database [17]. The probes designated for the experiments were composed of cDNA, a pair of specific primers and qPCRmix-HS SYBR+HighROX reagent (Evrogen). The total probe volume was 25 μl, the final concentration of the primers — 1 μM each and the expected cDNA concentration — 4 μg/ml. The experiments were carried out in 48-well plates (Illumina, USA) using the Eco real-time PCR system (Illumina). The annealing temperature was set to 60 °C. Prior the experiment, the plates were sealed with a transparent film and centrifuged (100 g, 3 min, 18 °C) to prevent uneven probe distribution. The data were analyzed using the software supplied by Illumina. The *ACTB* assay was used as an endogenous control. Each probe was run in triplicates. After all, three independent experiments were performed.

Proliferation assay

For proliferation assay, the cells were seeded in 6-well plates, 40,000 cells per well. Randomly selected samples were treated with 0.25 % of trypsin-EDTA solution (PanEco) on a daily basis. The cells were counted in a hemocytometer. The obtained data were used to plot cell growth curves in Cartesian coordinates. Each experiment was repeated for 3 times.

Scratch assay

To estimate the rate of cell migration, the cells were cultured until they covered the entire dish surface. Before the experiment, the cell monolayer was scratched with a pipette tip to form a 1.25 mm-wide cell-free area across the center of the well. Then, the remaining cells were cultured for 5–6 days. The cell-free areas were photographed daily and quantified using Freehand selection tool in the ImageJ menu.

Statistical analysis

Data were represented as mean ± standard deviation (m ± SD). The statistical differences between the means were assessed by a one-way ANOVA. If P-values were less than 0.05, means were considered to be significantly different.

RESULTS

Preparation of lentiviral particles

Fluorescence of transfected HEK293 became evident the next day after transfection. Then, the ratio of fluorescent cells increased to 75–85 % (Fig. 1A). Besides, we discovered that the transfection with the vector encoding the IC-specific shRNA affected cell adhesion to the growth surface. Unlike the cells transfected with the vector that encoded the scramble shRNA (HEK293-CTR), the cells transfected with the vector that encoded the shRNA (HEK293-IC) remained attached to the growth surface even when the cells were cultured for a long time and the culture medium became more acidic. Moreover, HEK293-IC required a treatment with trypsin before reseeding, whereas HEK293-IC could be resuspended with an automatic pipette.

Transduction of epidermal keratinocytes and their selection with puromycin

Fluorescence in the transduced HaCaT cells became evident on the 2nd day of transduction. At the mentioned time point, the ratio of fluorescent cells in the samples did not exceed 10 % (Fig. 1B). On days 3 and 4, the ratio of fluorescent HaCaT cells reached 35–50 %.

To remove uninfected cells from the samples, the transduced cells were transferred to the culture medium supplemented with puromycin. For the first 3 days of culturing, the number of cells dropped dramatically. The non-transduced cells, which served us as a negative control, did not survive in the presence of antibiotic (Fig. 1C). In the other samples, the ratios of viable cells were 15–25 % of the cells treated with puromycin.

Moreover, we demonstrated that cells expressing the IC-specific shRNA (HaCaT-IC) and those expressing scramble shRNA (HaCaT-CTR) exhibited different morphological characteristics. Specifically, the colonies of HaCaT-IC cells exhibited sharp boundaries (Fig. 2A). Besides, multiple HaCaT-IC cells grew on the top of each other suggesting that the intercellular contacts between these cells are stronger, compared to HaCaT-CTR (Fig. 2B). In contrast, HaCaT-CTR cells retained the morphological characteristics typical for non-transduced cells: the boundaries of their colonies were blurred, and, unlike HaCaT-IC, they formed a regular monolayer once confluency had been reached.

Changes in gene expression profile of transduced cell

In epidermal keratinocytes, IC silencing led to a 20-fold reduction in the expression of the IC encoding gene (*MMP1*). In contrast, the quantitative PCR did not reveal statistically significant differences in the expression of metalloproteinase genes *MMP2*, *MMP9* and *MMP12* (Fig. 3A). At the same time, we observed shifts in the expression of *IVL* and *FLG* (0.79 ± 0.11 and 6.99 ± 0.97, respectively, Fig. 3B). The protein products of these genes (involucrin and filaggrin, respectively) play a key role in the differentiation of epidermal keratinocytes. Moreover, the cells lacking IC demonstrated an aberrant expression of cytokeratins characteristic for healthy and psoriatic epidermis (Fig. 3C). For example, the expression of *KRT1* and *-14* in HaCaT-IC cells was increased 16.89 ± 0.97 and 2.36 ± 0.19 times, respectively, whereas the expression of *KRT17* and *-18* went down to 0.12 ± 0.01 and 0.56 ± 0.02, respectively.

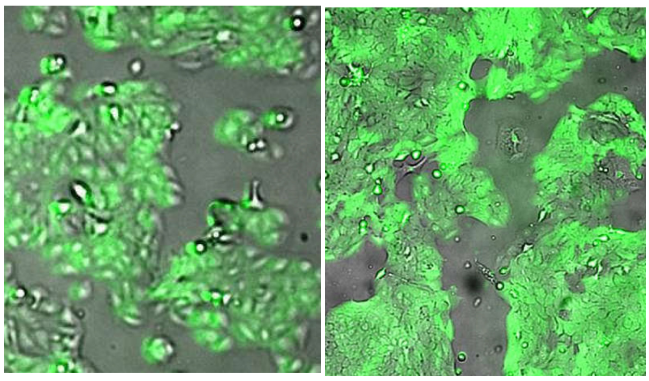

Fig. 2. Effect of mRNA expression on the morphological characteristics of transduced cells. **(A)** Cells expressing control shRNA. **(B)** Cells expressing shRNA specific to interstitial collagenase. Transduction of HaCaT cells was performed as described in *Methods*

Proliferation and migration of transduced cells

Analysis of the cell growth did not reveal any significant differences in the proliferation rates of HaCaT-IC and HaCaT-CTR cells (Fig. 4A). In contrast, analysis of cell migration revealed that knocking IC down dramatically affected the mobility of HaCaT-IC cells. In this respect, the migration of HaCaT-IC resulted in a reduction of the injured area by ~40 %, whereas HaCaT-CTR cells reduced it by ~85 % on day 5 of the experiment (Fig. 4C).

Enzymatic activity of IC in the transduced cells

Assessment of IC enzymatic activity by densitometry revealed that both cell lines were capable to produce collagenases IC, MMP2 and MMP9 as well as secrete these enzymes into the medium (Fig. 5A and B). However, the level of secreted IC produced by HaCaT-IC cells was 4 times lower, compared to HaCaT-CTR (Fig. 5C). Moreover, analysis of cell homogenates revealed that HaCaT-CTR cells also produced small amounts

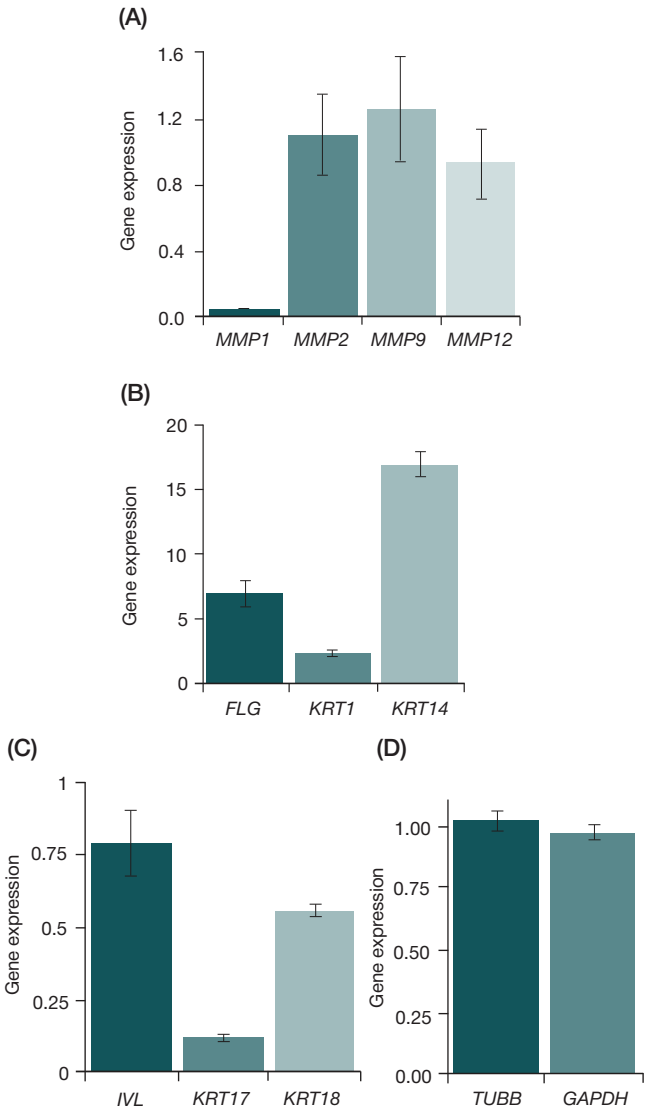

Fig. 3. Assessment of gene expression in transduced human epidermal keratinocytes by quantitative PCR. **(A)** Shifts in matrix metalloproteinase expression. **(B)** and **(C)** Shifts in over-and underexpressed genes. **(D)** Housekeeping gene expression. Data were normalized to the expression of *ACTB*. The figure shows results of comparison of gene expression in epidermal keratinocytes expressing interstitial collagenase-specific shRNA and control shRNA (see *Methods*)

of pro-MMP1, which was undetectable in the homogenates obtained from HaCaT-IC cells (Fig. 5B and C).

DISCUSSION

In this study, we generated two cell lines of immortalized human epidermal keratinocytes. One of them (HaCaT-CTR) expressed scramble shRNA and it was used as a control. Another one (HaCaT-IC) expressed the IC-specific shRNA. We compared morphological characteristics of both cell lines (Fig. 2). We also showed that the expression of IC-specific shRNA significantly affected cell migration (Fig. 4C). However, it did not have a significant influence on the proliferation rate (Fig. 4A). Moreover, we revealed that IC-silencing caused changes in the expression of cytokeratins (*KRT1*, *-14*, *-17*, *-18*), involucrin (*IVL*) and filaggrin (*FLG*). In the other words, it primarily affected the genes linked to the differentiation of epidermal keratinocytes (Fig. 3).

Notably, culturing the transduced cells revealed that HaCaT-IC and HaCaT-CTR had different morphological characteristics. Particularly, the colonies of HaCaT-IC cells exhibited sharp boundaries, where multiple cells were growing on the top of the others (Fig. 2A). In contrast, HaCaT-CTR retained the appearance typical for non-transduced cells. The boundaries of their colonies were blurred. Moreover, when HaCaT-CTR reached confluency, they formed a monolayer (Fig. 2B). Based on the fact that difference in HaCaT-CTR and HaCaT-IC genotypes were caused by the shRNA encoding transgene, we concluded that different morphological appearance of HaCaT-CTR and HaCaT-IC cells could be only explained by IC silencing.

According to the published data [2], IC regulates cell adhesion and also contributes to cell migration that follows the injury. The cells expressing IC at a high level, such as invasive cancer cells, poorly interact to each other and tend to migrate. In turn, silencing IC in these cells makes them to interact to each other and reduces their migration rate. Consequently, it reduces the risk of metastasis that may occur in the distal organs and tissues of experimental animals [18]. In this regard, the results of our study are consistent with the published report. As we discovered, the intercellular contacts between HaCaT-IC cells are stronger and their migration rate is 2.5 fold less, compared to HaCaT-CTR cells (Fig. 4B and C). Notably, we also observed similar changes in HEK293-IC cells. Particularly, we noticed that HEK293-IC cells are stronger attached to the growth surface, compared to HEK293-IC.

Taking in account the structure of the epidermis, we have to notice that the ability to migrate is strongly restricted to undifferentiated and poorly differentiated keratinocytes of the basal layer. In contrast, the mobility of epidermal keratinocytes residing in the other layers is quite limited because these cells are at the later stages of the terminal differentiation and they are held together through a set of intercellular contacts, such as desmosomes. However, the number of the cells capable to migrate significantly increases during the development of psoriatic plaques due to epidermal hyperplasia. In this respect, we assume that morphological changes in HaCaT-IC cells could not be only caused by the IC-deficiency but their progression through the terminal differentiation program.

As shown earlier, manipulations with a single gene, such as superexpression or knockout, can result in dramatic consequences for the entire organism causing misregulation of at least several hundred genes [20, 21]. In contrast, silencing the same gene by specific shRNA may gradually adjust its

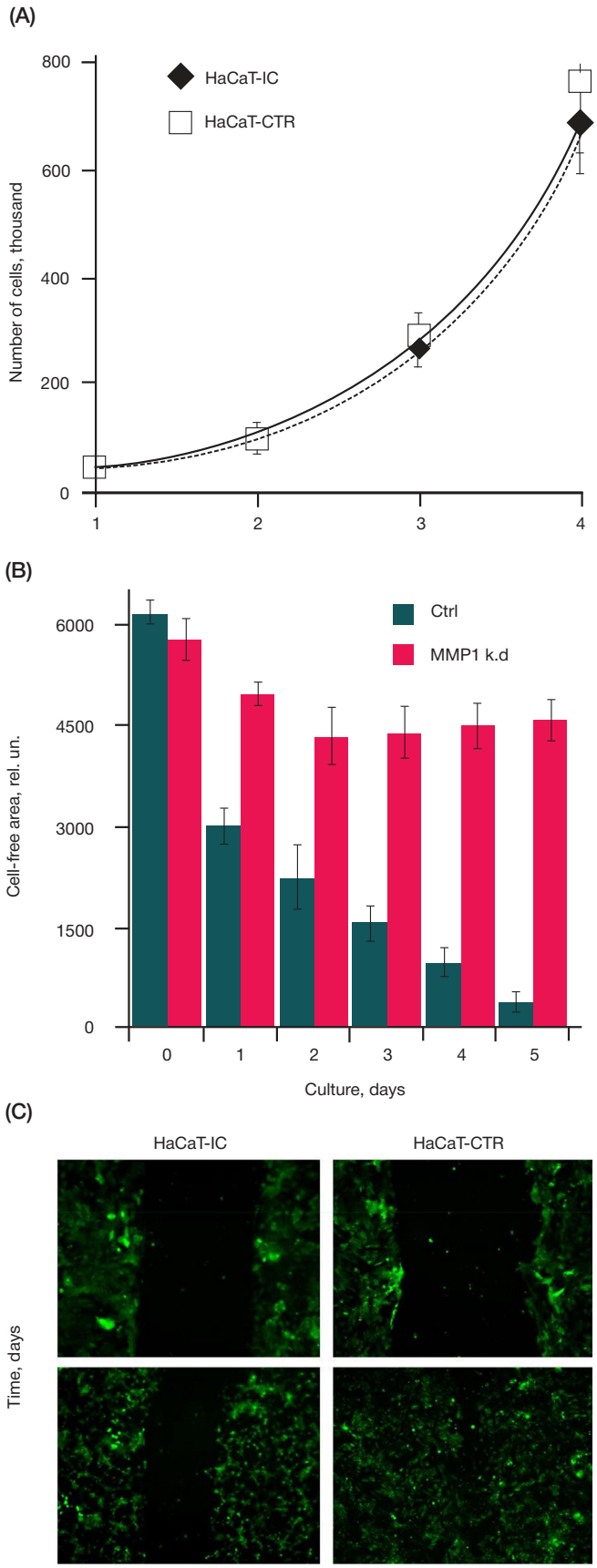

Fig. 4. Effect of expression of in interstitial collagenase-specific shRNA on proliferation and migration of transduced cells. Analysis of cell growth curves **(A)** and quantitative assessment of migration rate **(B)** of HaCaT cells expressing IC-specific shRNA and scramble shRNA. **(C)** Migration of transduced cells: photos taken the next day after transduction began and after it was finished. For details please refer to *Methods*

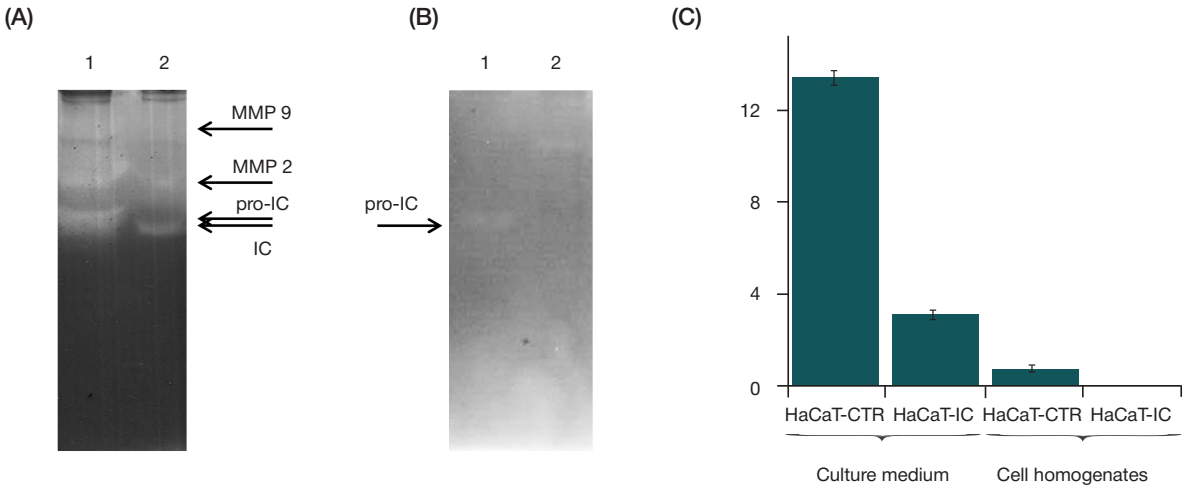

Fig. 5. Analysis of interstitial collagenase activity in transduced epidermal keratinocytes, cell homogenates and culture medium. **(A)** Zymography of culture medium samples collected from growing transduced cells: 1 — cells expressing scramble shRNA. 2 — cells expressing IC-specific shRNA. The arrows show positions of matrix metalloproteinases in the gel. **(B)** Zymography of homogenate samples obtained from growing transduced cells: 1 — cells expressing scramble shRNA. 2 — cells expressing IC-specific shRNA. **(C)** Quantitative analysis of enzymatic activity. Samples were collected 48 hours after culture onset. Confluency at the time of sample collection — 60 %. Cell culturing, preparation of homogenates, details of protein electrophoresis and zymography are described in *Methods*

expression to the required level if nucleotide sequences with various affinities to the specific mRNA are tried. Alternatively, the same result can be achieved by using an appropriate number of viral particles for the transduction. In this respect, a comparative analysis of gene expression in HaCaT-CTR and HaCaT-IC cells demonstrated that a 20-fold reduction in the expression of MMP1 did not change the expression of homologous matrix metalloproteinases — *MMP2*, *-9*, and *-12* (Fig. 3A).

An absence of statistically significant changes in the expression of these genes is important for at least two reasons. First, it means that we managed to block the IC biosynthesis selectively, i. e. we did not disturb the expression of the homologous genes that could be targeted by the specific IC shRNA in the first turn. Second, the obtained results are relevant to the specific phenotypical changes that we observed in HaCaT-IC cells (Fig. 2).

In the same time, assessment of total collagenase activity by densitometry demonstrates that IC activity in the culture medium collected from HaCaT-IC cells is only 4-fold less, compared to HaCaT-CTR cells (Fig. 5B). However, we did not observe any significant accumulation of pro-IC in the cytoplasm (Fig. 5B). As we believe, more prominent changes in *MMP1* expression (Fig. 3A) compared to IC activity (Fig. 5B) are caused by overloading the protein samples that we used for zymography. In this respect, the band intensities could grow proportionally up to a certain point unless the substrate was not completely exhausted.

Moreover, a comparative analysis of gene expression in HaCaT-CTR and HaCaT-IC cells revealed statistically significant differences in the expression levels of cytokeratins *KRT1*, *-14*, *-17*, and *-18*. Specifically, we found that *KRT1* and *-14* were upregulated, whereas *KRT17* and *-18* were downregulated (Fig. 3B). According to the literature, up- or downregulation of these genes are of great physiological and clinical importance. Particularly, the maintenance of normal expression levels of *KRT1* and *-14* in healthy epidermis is crucial for the normal flow of the terminal differentiation of epidermal keratinocytes [22]. On the other hand, the development of psoriatic plaques that occurs due to a disturbance of the terminal differentiation program is accompanied by downregulation of *KRT1* and *-14* and upregulation of *KRT17* and *-18* [23, 24]. In this respect (Fig. 3C), our results indicate that knocking IC down may have an important therapeutic effect in psoriasis, because it partially normalizes the expression of the mentioned cytokeratins, i.e. it reduces the expression of *KRT17* and *-18* as well as stimulates the expression of *KRT1* and *-14*.

Besides, IC silencing alters the expression of *IVL* and *FLG* (Fig. 3B). To the reference, proteins encoded by these genes (involucrin and filaggrin, respectively) are the important structural components of the cornified envelop. They are also used as biomarkers that help to distinguish between early and late stages in the terminal differentiation of epidermal keratinocytes. In turn, the differential expression of mentioned genes in psoriasis causes structural rearrangements in the cornified envelop, which result in the development of psoriatic plaques. Particularly, *IVL* becomes upregulated, whereas *FLG* — downregulated [25]. In this respect, our results indicate that IC silencing may have a therapeutic effect in psoriasis (Fig. 3B), because it partially normalizes *IVL* and *FLG* expression in the transduced cells (Fig. 3B).

Importantly, the expression of *TUBB* and *GADPH*, which are frequently used as “housekeeping genes” in the other real-time PCR studies, did not change significantly (Fig. 3D) unlike the expression of other genes that we already discussed above (Fig. 3A–C). Based on these results (Fig. 3D), we suggest that *ACTB* can be used to normalize the real-time PCR data obtained for HaCaT-CTR and HaCaT-IC cells.

CONCLUSIONS

In conclusion, we would like to acknowledge that we obtained two new cell lines of epidermal keratinocytes — HaCaT-IC that expressed shRNA specific to IC and HaCaT-CTR that expressed scramble shRNA. These cell lines had different morphological characteristics. Moreover, IC silencing affected the ability of HaCaT-IC cells to migrate. The comparative analysis of gene expression revealed that knocking IC down in epidermal keratinocytes could become a promising therapeutic tool that to normalize the expression of *IVL*, *FLG*, *KRT1*, *-14*, *-17*, and *-18* in lesional psoriatic skin.

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